Photogallery

In situ observation of Denise's pygmy seahorse Hippocampus denise associated with a gorgonian coral Annella reticulata at Osprey Reef, Australia

Jun NISHIKAWA^{1,*}, Richard FITZPATRICK², James D. REIMER³, Robin J. BEAMAN², Hiroyuki YAMAMOTO⁴, and Dhugal J. LINDSAY⁴

- ¹ Atmosphere and Ocean Research Institute, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan
- ² James Cook University, McGregor Road, Smithfield, Cairns, Qeensland 4870, Australia
- ³Transdisciplinary Research Organization for Subtropics and Island Studies, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903–0213, Japan
- ⁴ Japan Agency for Marine-Earth Science and Technology, 2-15, Natsushima, Yokosuka, Kanagawa 237-0061, Japan

* Corresponding author: J. Nishikawa

E-mail: jn@aori.u-tokyo.ac.jp

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Hippocampus denise Lourie and Randall, 2003, one of the world's smallest seahorses, was described from Indonesia and appears to be relatively widespread in the West Pacific (Lourie and Randall 2003). The widespread occurrence of this species has been recorded in books, dive magazines and on the internet (e.g. Kuiter 2000), and recently the occurrence of this species was reported from Holmes Reef (Coral Sea) based on samples collected, together with gorgonians Villogorgia sp. from a depth of $\sim 100 \, \mathrm{m}$ (Foster et al. 2012). Biological and ecological data on this species are, however, very limited as well as $in \, situ$ observations of this species at such great depths.

Underwater observations using a high-definition video camera on the untethered remotely operated vehicle, PICASSO-1 (Fig. 1a), enabled us to confirm the occurrence of this species at Osprey Reef, a Coral Sea atoll in Australian waters, the first *in situ* record from this isolated oceanic reef, and even to measure breathing rates (2–5 Hz) and swimming distances (2.3–4.5 x body length) (see supplementary video footage). While no specimens of the observed seahorses were sampled, it is clear that the species we observed has a very low number of tubercles on its ventral region, and has an orange body color (see Fig. 1c). Based on these characters, the fishes were identified as *H. denise*. Three individuals (1 male and 2 females) were found on the branches of a gorgonian, *Annella reticulata* (Ellis and Solander, 1786), of size 1.72×1.53 m (Fig. 1b) on the steep reef wall near a prominent point of Osprey Reef [13.888368°S, 146.555328°E] at a depth of 102 m (water temperature 23.7°C), the deepest record of this species to date in the literature. This depth is similar to that reported in Foster et al. (2012) at Holmes Reef, suggesting that the occurrence of *H. denise* at such extreme depths may not be uncommon. *Hippocampus denise* was observed to only occur on *A. reticulata* among several species of gorgonians observed. The standard length (SL, as in Lourie and Randall 2003) measured from framegrabs of the fish was 21.5 mm for a "pregnant" male (Fig. 1c).

The approach of non-destructive sampling through the use of high-definition video allows both identification and behavioral observations to be made *in situ* in the mesophotic environment over prolonged periods. Future genetic examinations of this species will provide a clearer understanding of how such a host-specific and diminutive seahorse is able to settle and maintain their populations at

isolated oceanic reefs.

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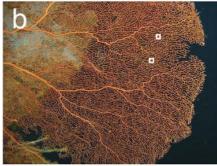
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Online Supplementary Material. A movie is available at http://www.jstage.jst.go.jp/browse/galaxea.

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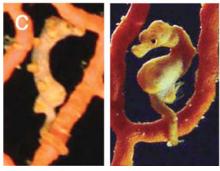


Fig. 1 a The remotely operated vehicle, PICAS-SO-1 used in this study. **b** A host gorgonian, *Annella reticulata*. White squares indicate the position of the seahorse framegrabs. See supplementary video footage. **c** *Hippocamus denise* video-recorded *in situ* on the host gorgonian. Left: female. Right: male